

We claim:

1. A method of crystallization, comprising the steps of:
 - a. introducing a first carrier-fluid into a first channel of a substrate;
 - b. introducing into a plug-forming region of the first channel:
 - 1) a first plug-fluid comprising a precipitant;
 - 2) a second plug-fluid comprising water; and
 - 3) a third plug-fluid comprising a crystallization target;where each of the plug-fluids comprises a solvent;
where pressure is applied when introducing each of the first, second and third plug-fluids, thereby resulting in the formation of a plurality of plugs in the first channel; and
where the crystallization target forms a crystal in at least one of the plugs.
- 15 2. The method of claim 1, further comprising the steps of:
 - a. introducing a second carrier-fluid into a second channel of the substrate;
 - b. introducing into a plug-forming region of the second channel:
 - 1) a first plug-fluid comprising a precipitant;
 - 2) a second plug-fluid comprising water; and
 - 3) a third plug-fluid comprising a crystallization target;where each of the plug-fluids comprises a solvent;
where pressure is applied when introducing each of the first, second and third plug-fluids, thereby resulting in the formation of a plurality of plugs in the channel;
where the first and second channels merge into a common downstream channel;
where at least one plug flowing from the first channel merges with a plug flowing from the second channel upon entry into the downstream channel.
 - 30 3. A method of crystallization, comprising the steps of:

- a. introducing a first carrier-fluid into a first channel of a substrate;
- b. introducing one or more first plug-fluids into a first plug-forming region of the first channel to form a plug of a first plug type; where a plug of the first plug type comprises a precipitant and a crystallization target;
- c. introducing one or more second plug-fluids into either one of:
- 1) the first plug-forming region;
 - 2) a second plug-forming region within the first channel; or
 - 3) a second plug-forming region within a second channel; where each of the plug-fluids comprises a solvent; where the one or more second plug-fluids form a plug of a second plug type at either one of the first, second and third plug-forming regions; where a plug of the second plug type comprises a precipitant; where pressure is applied when introducing the one or more first plug fluids and the one or more second plug fluids;
- where at least one or more plugs of the first plug type alternate in sequence with one or more plugs of the second plug type; and where the crystallization target forms a crystal in at least one of the plugs.
4. The method of claim 1 or 3, where the crystallization target is a member of the group consisting of protein, peptide, polynucleotide, oligonucleotide, subcellular organelle, subcellular protein complex, drug, small molecule/biological macromolecule complex, virus, colloidal particle, nanoparticle and combinations thereof.
5. The method of claim 1 or 3, where the crystallization target is a protein.

6. The method of claim 1 or 3, where there is transfer of a solvent from one plug into another plug.

7. The method of claim 6, where the solvent is water.

5 8. The method of claim 1 or 3, where water is transported from one plug into another plug.

9. The method of claim 3, where the one or more second plug-fluids is introduced into the first plug-forming region.

10 10. The method of claim 3, where the one or more second plug-fluids is introduced into the second plug-forming region.

11. The method of claim 3, where the one or more second plug-fluids is introduced into the second channel within the third plug-forming region.

12. The method of claim 3, where the first and second channels merge into a common downstream channel, such that one or more plugs flowing from the first channel alternate with one or more plugs of the second plug type flowing from the second channel upon entry into the downstream channel.

13. The method of claim 9, where a second carrier-fluid is introduced into the plug-forming region of the first channel so that it separates the one or more first plug-fluids from the one or more second plug-fluids prior to formation of the plug at the first plug-forming region.

20 14. The method of claim 13, where the second carrier-fluid is introduced through an inlet positioned between a first set of one or more inlets used for introducing one or more of the first plug-fluids and a second set of one or more inlets used for introducing one or more of the second plug-fluids.

25 15. The method of claim 1 or 3, where each of the carrier-fluids and plug-fluids is introduced into the substrate according to a given flow rate under pressure, either directly into a channel or through an inlet feeding into a channel.

16. The method of claim 15, where the pressure is applied continuously.

17. The method of claim 15, where the pressure is halted after one or more plugs are formed.

18. The method of claim 15, where at least one of the flow rates is constant relative to one another flow rate.

5 19. The method of claim 15, where at least one of the flow rates is varied relative to one another flow rate.

20. The method of claim 15, where at least one of the plug-fluids is introduced into a carrier-fluid at a constant flow rate.

10 21. The method of claim 15, where at least one of the plug-fluids is introduced into a carrier-fluid at a variable flow rate.

22. The method of claim 15, where the flow rates are varied resulting in a plurality of plugs exhibiting a concentration gradient with regard to one or more of the plug-fluids.

15 23. The method of claim 1 or 3, where mixing of the plug-fluids within the plugs is increased by adding a turn to a channel.

20 24. The method of claim 3, where each of the carrier-fluids and plug-fluids is introduced into the substrate according to a given flow rate under pressure, either directly into a channel or through an inlet feeding into a channel; and where one or more of the flow rates are varied so that the concentration of a first component in the plug of one plug-type provides a correlative and quantitative measure of a second component in an adjacent plug.

25. The method of claim 24, where the first component is a marker.

26. The method of claim 1 or 3, where at least one of the plug-fluids comprises a salt.

25 27. The method of claim 3, where the osmotic pressure in the plug of the first plug type at the first plug-forming region is lower than the osmotic

pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

28. The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 2 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

29. The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 5 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

30. The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 10 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

31. The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 50% of the plugs of the first plug type are adjacent to plugs of the second plug type.

32. The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 65% of the plugs of the first plug type are adjacent to plugs of the second plug type.

33. The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 95% of the plugs of the first plug type are adjacent to plugs of the second plug type.

34. The method of claim 3, where a plug flowing pattern is obtained in which each of the plugs of the first plug type is adjacent to a plug of the second plug type.

5 35. The method of claim 1 or 3, where at least one of the carrier-fluids comprises an oil.

36. The method of claim 1 or 3, where at least one of the carrier-fluids comprises a fluorinated compound.

37. The method of claim 1 or 3, where at least one of the carrier-fluids comprises a surfactant.

10 38. The method of claim 1 or 3, where at least one of the carrier-fluids is permeable to a component in a plug.

39. The method of claim 38, where the component is selected from the group consisting of water, acid, base, buffer and solvent.

15 40. The method of claim 1 or 3, where at least one of the carrier-fluids is permeable to water.

41. The method of claim 3, where the first and second carrier-fluids are permeable to water.

42. The method of claim 3, where the first and second carrier-fluids are the same.

20 43. The method of claim 3, where the first and second carrier-fluids are the different.

44. The method of claim 3, where at least one of the carrier-fluids is impermeable to water.

25 45. The method of claim 1 or 3, where at least one of the carrier-fluids comprises poly-3,3,3-trifluoropropylmethysiloxane (FMS-121).

46. The method of claim 1 or 3, where one of the carrier-fluids comprises perfluoroperhydrophenanthrene (PFP).

47. The method of claim 1 or 3, where one of the carrier-fluids comprises perfluoroperhydrophenanthrene (PFP) and 1H,1H,2H,2H-perfluorooctanol (PFO).

48. The method of claim 1 or 3, where the one of the carrier-fluids comprises 1H,1H,2H,2H-perfluorooctanol (PFO).

49. The method of claim 1 or 3, where at least one plug-fluid comprises a marker.

50. The method of claim 40, where the marker is an absorption dye or a fluorescent dye.

51. The method of claim 1 or 3, further comprising detecting the presence of a crystal in a plug.

52. The method of claim 1 or 3, further comprising analyzing the crystal in the capillary tube.

53. The method of claim 1 or 3, where a crystal is removed from a channel.

54. The method of claim 53, where the crystal is collected in a centrifuge tube.

55. The method of claim 53, where the crystal is collected in a sample tube.

56. The method of claim 53, where the crystal is collected in a micropipette.

57. The method of claim 53, where the crystal is collected in a capillary tube.

58. The method of claim 53, where the crystal is collected in a capillary tube and one or both ends of the capillary tube are sealed.

59. The method of claim 53, where the crystal is first collected in a capillary tube and then removed from the tube.

5 60. The method of claim 1 or 3, where the substrate comprises a material which allows water evaporation from the plugs.

61. The method of claim 1 or 3, where the substrate is soaked in water prior to crystal formation.

10 62. The method of claim 1 or 3, where the substrate is soaked in an aqueous salt solution prior to crystal formation.

63. The method of claim 1 or 3, where the substrate comprises a material which does not allow water evaporation from the plugs.

64. A method of indexing a component in the plug of a microfluidic substrate, comprising the steps of:

15 a. introducing a first carrier-fluid into a first channel of a substrate;

b. introducing one or more first plug-fluids into a first plug-forming region of the first channel to form a plug of a first plug type;

20 where a cross section of a plug of the first plug type formed is substantially the same size as a cross section of the first channel;

c. introducing one or more second plug fluids into either one of:

1) the first plug-forming region;

2) the second plug-forming region within the first

25 channel; and

3) a second plug-forming region within a second channel;

where the one or more second plug-fluids form a plug of a second plug type at either one of the first, second and third plug-forming regions;

- where second plug comprises a marker;
- where a cross section of plug of the second type formed is substantially the same size as a cross section of the channel in which it is formed;
- where pressure is applied when introducing the one or more first plug fluids and the one or more second plug fluids, thereby inducing a flow of plugs in the substrate;
- where at least one or more plugs of the first plug type alternate in sequence with one or more plugs of the second plug type;
- where each of the carrier-fluids and plug-fluids is introduced into the substrate according to a given flow rate under pressure, either directly into a channel or through an inlet feeding into a channel; and
- where one or more of the flow rates are regulated so that the marker provides a correlative and quantitative measure of a component in an adjacent plug.
- 15 65. The method of claim 1, 3 or 64, where the second plug fluid further comprises a dehydration agent.
66. A capillary tube comprising a plurality of plugs in an immiscible carrier-fluid, where a cross section of each plug is substantially the same size as a cross section of the capillary tube, said plurality of plugs comprising at least one plug having a crystallized target substance.
- 20 67. The capillary tube of claim 66, where the crystallization target is a member of the group consisting of protein, peptide, polynucleotide, oligonucleotide, subcellular organelle, subcellular protein complex, drug, small molecule/biological macromolecule complex, virus, colloidal particle, nanoparticle, and combinations thereof.
- 25 68. The capillary tube of 66, where the crystallization target is a first protein.
69. The capillary tube of claim 68, where the first protein is bound to a synthetic molecule or a small molecule.

70. The capillary tube of claim 68, where the first protein is bound to a drug.

71. The capillary tube of claim 68, where the first protein is bound to a second protein or peptide.

5 72. The capillary tube of claim 68, where the first protein is bound to a member of the group consisting of oligonucleotides, carbohydrates and sugars.

73. The capillary tube of claim 68, where the carrier-fluid comprises an oil.

10 74. The capillary tube of claim 68, where the carrier-fluid comprises a fluorinated compound.